(54) Title: IMPROVED STABLE COAGULATION CONTROLS

(57) Abstract

Improved stable coagulation control plasmas are described which contain blood plasma, and effective amounts of (a) a buffer to maintain a physiological pH, (b) a protease inhibitor, and (c) a suitable stabilizing carbohydrate. Preferred control plasmas also contain thimerosal and/or sodium azide. Also described are stable control plasmas having high levels of Factor V and VIII including plasma from blood which has been collected directly from an animal source into a solution containing (a) a buffer to maintain a physiological pH, (b) a protease inhibitor, and (c) citrate, and to which, after removal of red blood cells, has been added at least about 2 weight percent of a suitable stabilizing carbohydrate, such as sucrose. Methods for preparing stable control plasmas are also described.

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## IMPROVED STABLE COAGULATION CONTROLS

### BACKGROUND OF THE INVENTION

The present invention relates generally to blood coagulation, and more particularly to improved blood

5   coagulation controls which demonstrate superior stability, and also sensitivity to variation in reagents used in coagulation tests.

The need for stable and reliable blood coagulation controls is well documented. The continued and increased

10  use of oral anticoagulants for treatment and management of various thrombo-embolytic conditions today, more than ever, is a driving force for their development. As is known, overdosage of anticoagulants, commonly the coumarin derivatives, can lead to serious complications, including

15  hemorrhage from peptic ulcers, as well as other gastrointestinal complications. On the other hand, maintenance of too low a level of anticoagulant reduces or eliminates the efficacy of the prescribed treatment.

It is therefore extremely important that anticoagulant

20  levels be reliably monitored, and, as such, a voluminous body of art has developed documenting attempts of those in the field to produce stable and reliable controls, as well as reagents, to aid in monitoring anticoagulant activity.

For example, U. S. Patent No. 3,947,378 to Babson

25  discloses a process for producing a control plasma deficient in Factors II, VII, IX, and X which involves treating to plasma with 20 to 22% by weight of barium sulfate at ambient temperature and then removing the adsorbent from the adsorbed plasma. The Babson patent reports that abnormal

30  control plasmas produced by mixing the so-adsorbed plasma with normal plasma are more stable after reconstitution and give more uniform results in the activated partial thromboplastin time (APTT) procedure after storage of up to eight hours or more.

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S. Zucker, M.H. Cathey, and B. West, *Preparation of Quality
Control Specimens for Coagulation*, Amer. J. Clin. Path., June 1970,
Vol. 53 pp. 924-927, reports a method for preparing
lyophilized plasma specimens for use as quality controls in
5      coagulation testing.   Zucker et al. report buffering the
plasma specimens with
N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES),
which was reported to provide pH and enzyme stability for
prothrombin times for eight hours at 25°C.   An article by M.
10    Brozovic, D. J. Howarth, L. P. van Halem Visser, and E. A.
Loeliger, *Stability of Freeze-Dried Plasma Prepared from Patients on Oral
Anticoagulants*, Journal of Clinical Pathology, 1973, Vol. 26,
p. 857-963, reported as to the suitability of freeze-dried
plasmas from patients on oral anticoagulants to serve as
15    reference material in the calibration of thromboplastins
used in the control of oral anticoagulant treatment.   The
authors studied plasmas from several plasma pools, developed
by collecting from each patient 4.5 ml of blood into 0.5 ml
of a solution formed by combining 44.62 g of HEPES buffer,
20    38.00 g trisodium citrate ($2H_2O$), and 0.5 ml aprotinin
TRASYLOL (1000 u/ml), and then adding distilled water to
1000 ml.   Individual samples were centrifuged, as were the
subsequently pooled samples, whereafter 1 ml of 10% sodium
azide was added per liter of plasma.   Samples of the
25    prepared plasmas were then either frozen or freeze-dried.
The authors reported that the freeze-dried plasmas from
patients on oral anticoagulants could be used to calibrate
thromboplastins provided that they are used immediately
after complete reconstitution or kept at 4°C for use within
30    four to six hours of reconstitution.   The authors also
reported that their plasma samples generally demonstrated
varying levels and types of instability after reconstitution
and storage at 4°C., 22°C., and 37°C.   It was generally
reported that this instability resulted after about
35    twenty-four hours at the lower two

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temperatures and after about four to six hours at the higher
37°C temperature.

United States Patent No. 4,007,008 to Becker et al.
describes a method for reducing enzyme activities in animal
5    serum or plasma including the steps of raising the pH
thereof to a level about that of normal serum by adding a
base, and thereafter terminating the reaction or reduction
of enzyme activities neutralizing the serum or plasma with
an acidic medium.

10       Other general background can be found in United States
Patent No. 3,799,885 to Dennis et al. which discloses a
calcium chloride test reagent buffered with HEPES buffer
which is especially adapted for use in monitoring heparin
therapy; in United States Patent No. 4,301,028 to Bartl et
15   al. which reports a control reagent for heparin activity
determination; in United States Patent No. 4,116,336 to
Sorenson et al. which relates to a package containing a
synthetic reference liquid for quality control and/or
calibration of blood gas measuring equipment; and in P. S.
20   Roberts, H. N. Hughes, and P. B. Fleming, *The Effects of Hepes*
*Buffer on Clotting Tests, Assay of Factors V and VIII and on the Hydrolysis of Esters*
*by Thrombin and Thrombokinase,* Thrombos, Haemostas, (Stuttg.),
1976, Vol. 35, p. 202, wherein the authors report faster
clotting in the presence of 50 mM HEPES buffer.

25       Another aspect of the prior art control plasmas is that
a majority of them, especially those commercially available,
consist of or otherwise comprise primate plasma, most
commonly human.  These plasmas present disadvantages in that
they contain unstable human factors, particularly Factors V
30   and VIII, and also present a greater risk to the preparer or
user of the controls, since they may harbor active AIDS or
hepatitis viruses.

In the face of the voluminous literature and other work
relating to plasmas for coagulation controls, there still

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remains a need for a coagulation control which exhibits
superior stability with respect to one-stage prothrombin
times (PT), activated partial thromboplastin times (APTT),
and Factor V and VIII activity values, as well as superior
5  sensitivity to variations in clotting test reagents
employed.  Certain forms of improved controls would also
significantly eliminate risk of AIDS or hepatitis
contraction to those who prepare and use it.  The
applicant's invention, in its various aspects, addresses
10  these matters.

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## SUMMARY OF THE INVENTION

Accordingly, one preferred embodiment of the applicant's invention relates to a stable coagulation control plasma comprising blood plasma, and effective amounts of (a) a

5    buffer to maintain a physiological pH, (b) a protease inhibitor, and (c) a suitable stabilizing carbohydrate. In a preferred aspect the control has a total plasma component constituted substantially of non-primate plasma.

Another preferred embodiment of the applicant's

10   invention relates to a coagulation control plasma having high stabilized levels of Factor V and VIII. This control comprises plasma and at least about 2 weight percent of a suitable carbohydrate. The plasma of this embodiment is derived from blood which has been collected directly from an

15   animal source into a solution containing a buffer to maintain a physiological pH, a protease inhibitor, and citrate. The carbohydrate in the control is added after removal of red blood cells from the blood.

Another preferred embodiment of the applicant's

20   invention relates to a process for producing a stable coagulation control plasma. This process includes the sequential steps of collecting the blood from which the plasma is derived directly from an animal source into a solution containing a buffer to maintain physiological pH, a

25   protease inhibitor, and citrate; removing red blood cells from said blood; and adding a suitable stabilizing carbohydrate.

Still another preferred embodiment of this invention relates to a process for producing a stable coagulation

30   control which includes the steps of providing blood plasma; and, adding to said blood plasma at least one purified, stabilized coagulation factor.

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## DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTIONS

One preferred embodiment of the applicant's invention relates to a stable coagulation control plasma which comprises blood plasma, and effective amounts of (a) a

5    buffer to maintain a physiological pH, (b) a protease inhibitor, and (c) a suitable stabilizing carbohydrate.  It is contemplated that a carbohydrate as used herein would have ratios of H to O of 2 to 1, as is understood in the art.

As to types of plasmas which are suitable for this

10   embodiment, primate and non-primate plasmas can be used. Preferred plasmas to date have been human, bovine, porcine, equine and rabbit plasmas, although other plasmas including, for instance, goat and sheep plasmas, can be used. Additionally, in this embodiment of the invention, these

15   plasmas may be collected in any suitable manner as known in the art.

As to the composition of the blood plasma in the control, various animal plasmas may be used individually or combined, adsorbed or non-adsorbed.  In most cases, however,

20   the applicant has to date preferred combining the plasmas of two or more types of animal.  Since different animals have differing levels and differing stabilities of their respective coagulation factors, different animal plasmas can be selected and combined to adjust the ratios of coagulation

25   factors in the controls and thus also adjust stability of the control and clotting times of the PT, APTT, and other tests.

For instance, owing to their particular coagulation constituent levels, rabbit plasma is used to shorten PT

30   values, porcine plasma is used to shorten APTT but not PT values, bovine plasma is used to furnish high levels of factor V, and equine plasma is used to prolong the APTT but not the PT value.  By using these principles, the applicant has been able to prepare, as desired, control plasmas

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exhibiting particular ranges (e.g.. normal or abnormal) of
PT and APTT values. Clotting times can thus be precisely
adjusted according to the types and ratios of plasmas used.

Of the preferred control plasmas which have been
5    prepared to date, four have shown to be more preferred.
The first exhibits PT and APTT values in the normal range,
and has a total plasma component constituted of porcine,
bovine and rabbit plasma in ratios of about 1:6:3,
respectively. The second has PT and APTT values in the
10   abnormal range and has a total plasma component constituted
of porcine plasma adsorbed with aluminum hydroxide gel, and
bovine plasma in a ratio of about 35:1, respectively. The
third plasma also exhibits PT and APTT times in the abnormal
range, and has a total plasma component constituted of
15   porcine plasma adsorbed with aluminum hydroxide gel.

These first three more preferred plasmas provide the
advantage of significantly reduced risk of HIV or hepatitis
virus infection by those who prepare and/or use them since
the pertinent viruses are not present in the non-primate
20   plasmas. Additional advantages have related to high and
stable levels of coagulation factors these plasmas have
provided. In this regard, it is understood that similar
advantages can be derived so long as the non-primate plasma
constitutes at least a substantial part of the total plasma
25   component of the control, and thus such control plasmas are
also a preferred aspect of this invention, as are controls
whose plasma component consists essentially of non-primate
plasmas.

The fourth more preferred control has a plasma component
30   constituted of normal human, bovine, and rabbit plasma in
respective ratios of about 2:2:1.

With respect to the buffer, to date, HEPES buffer has
been preferred, although many other buffers, for instance
TRIS, are known and are suitable. The preferred HEPES
35   buffer has been HEPES hemi sodium, which is preferably

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present in the control in an amount of at least about 0.08
M, and preferrably above .05 in amount.

As to amounts and types of protease inhibitor and
carbohydrate, it has been preferred to date that the
inhibitor and carbohydrate be present in the control in
amounts of at least about 0.5 U/ml, and 2 weight percent,
respectively. The preferred protease inhibitor has been
aprotinin, although other such protease inhibitors, for
instance soya bean trypsin inhibitors, are known in the art
and are suitable. The preferred stabilizing carbohydrate to
date has been saccharides, and in particular, sucrose,
although others well known in the art can also be used.
Additionally, it has been more preferred to date that the
sucrose be present in the control in an amount of about 5
weight percent.

As to other components of the control plasma of this
preferred embodiment, it has also been preferred that the
control plasma include thimerosal in an amount of
preferrably at least about 0.02 weight percent and/or sodium
azide in an amount of preferrably at least about 0.08 weight
percent.

To formulate a batch of control plasma, approximately 1
part (by volume) of the applicant's preferred buffering and
preservative solution described in Example 1 below are
combined with about 9 parts plasma. More preferred to date
has been to combine approximately 1 part of the applicant's
preferred buffering preservative solution with about 9 parts
of mixed or unmixed non-primate animal plasma.

Further details of the preparation of the control
plasmas of this preferred embodiment, as well as details of
their stability, can be found in Examples 1-6 and Tables 1-3
below. Collectively, the control plasmas of this embodiment
have generally proven stable, meaning that APTT and PT
analyses do not change more than about 10% for at least
about 3 days at room temperature, or at least about 8 hours
at 37°C.

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Additionally, as is particularly detailed in Example 6
below, the coagulation controls of this preferred embodiment
have demonstrated increased sensitivity to variations in
reagents used in clotting time testing.  Example 6 details
5    experiments wherein a coagulation control sample prepared in
accordance with this embodiment was tested against a
commercially available plasma, CITROL I, and fresh normal
human plasma as regards sensitivity to dilutions of
thromboplastin reagent in the PT test.  As the reported
10   results demonstrate, the control prepared in accordance with
the applicant's invention herein demonstrated superior
sensitivity to the varying dilutions of thromboplastins.

As stated, another preferred embodiment of the present
invention involves a coagulation control plasma having high
15   stabilized levels of Factor V and VIII.  This control
comprises plasma derived from blood which has been collected
directly from an animal source into a collecting solution
containing a buffer to maintain physiological pH, a protease
inhibitor, and citrate, and to which, after removal of red
20   blood cells, has been added at least about 2 weight percent
of a suitable stabilizing carbohydrate.

Although others can be used, to date, the preferred
buffer into which the blood has been collected has been
HEPES hemi-sodium, and the preferred protease inhibitor has
25   been aprotinin.  It has also been preferred that the HEPES
hemi-sodium and the aprotinin be present in the collecting
solution in respective amounts of at least about 0.25 M and
5 U/ml.  Citrate can be present in the collecting solution
in suitable amount as known in the art.  To date, however,
30   it has been preferred that sodium citrate be present in an
amount of about 3 weight percent.  When these preferred
amounts of HEPES hemi-sodium, aprotinin, and citrate are
used, about 9 parts (by volume) blood are preferably
collected into about 1 part of the collecting solution.
35   The carbohydrate can be added in solid form or in

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solution. Again, the preferred carbohydrate has been
sucrose, which has been added after removal of red blood
cells from the plasma by centrifuging or another suitable
method. Additionally, it has been more preferred to add
5      sucrose to a level of about 5 weight percent in the
control. If desired, additional physiological pH buffer can
also be added (as a solid or in solution) to effect a
desired level in the final control. Preferably, the final
control has also contained thimerosal (preferably at least
10     about 0.02 weight percent) and sodium azide (preferably at
least about 0.08 weight percent). The thimerosal and/or
sodium azide can be present initially in the collecting
solution or can be added later as a solid or in solution.

The applicant has discovered that preferred plasmas
15     prepared according to this embodiment are very high in
Factor V and VIII content. Additionally, the applicant has
found that the Factor V and VIII contents thus produced are
highly stable at both room temperature (for at least about 3
days) and at about 37°C (for at least about 8 hours).

20     Plasmas which are suitable for this embodiment of the
invention include primate and non-primate plasmas, with
preferred plasmas being beef, pig, rabbit, horse, and human
plasmas. Additional details of the preparation of control
plasmas of this embodiment can be found in Examples 7-11
25     below.

Another preferred embodiment of the present invention
concerns a method for producing a stable coagulation
control. As stated above, this method includes the
sequential steps of (a) collecting the blood containing the
30     plasma to be used in the control directly from an animal
source into a solution containing a buffer effective to
maintain a physiological pH, a protease inhibitor, and
citrate, (b) removing red blood cells from said blood, and
(c) adding at least about 2 weight percent of a suitable
35     stabilizing carbohydrate. Further details of this preferred

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process are analogous to those discussed in the embodiment
immediately above, and can further be found in Examples 7-11
below.

5
Still another preferred embodiment of this invention
concerns a method for producing a stable coagulation control
plasma, which method involves the steps of providing blood
plasma, and adding to said plasma at least one stable
purified coagulation factor in order to increase the level
of the added factor in the plasma. As is further detailed
10
in Example 12 below, particular factors are purified from
various animal plasmas as known in the art. These purified
factors are then added to plasma to increase the level of
the added factor in the plasma and to adjust the PT and/or
APTT values. Preferred methods also involve adding to
15
equine or human plasma stable purified Factor V or VIII from
bovines or porcines. Additionally, purified equine Factor
II has proven particularly stable and can be added to other
plasmas as a source of stable Factor II. Collectively, the
plasmas to which the stable purified factors have been added
20
have demonstrated stability superior to similar plasmas
without the added factors.

Reference will now be made to specific Examples and
Tables for the purposes of further describing and
understanding the features of the applicant's preferred
25
embodiments as well as their advantages and improvements
over the art. It should be understood that these Examples
are representative only, and that such additional
embodiments and improvements of the same are within the
contemplation and scope of the applicant's invention as
30
would occur to one of ordinary skill in this art.

The Factor V and VIII activity values reported in the
following Examples were calculated in the conventional
manner against a standard curve prepared from dilutions of
normal human plasma. PT intends the one-stage prothrombin
35
time, and APTT intends the activated partial thromboplastin

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time.   The PT and APTT tests reported were performed in the
conventional manner.

## EXAMPLE 1
### Preparation of Preferred Preservative Solution

5      100 ml of the applicant's preferred preservative
solution were prepared by mixing 500 U aprotinin, about
12.5g HEPES hemi sodium, 0.1g thimerosal, 0.5g $NaN_3$, and
25g sucrose, and then adding distilled water to 100 ml.
This preparation resulted in 100 ml of preservative solution
10   which consisted of:
       (a)   0.5 M HEPES hemi sodium;
       (b)   0.1% thimerosal;
       (c)   25% sucrose;
       (d)   0.5% sodium azide; and
15     (e)   500 U aprotinin.


## EXAMPLE 2
### Non–Primate Based Normal Range Control (Level 1)

       A pilot lot (hereafter designated P1) of non–primate
based control was prepared which demonstrated PT and APTT
20   values in the normal range.  Particularly, 54 ml beef
plasma, 9 ml pig plasma, and 27 ml rabbit plasma were
blended with 10 ml of the preferred preservative solution of
Example 1.  The initial PT and APTT values were 11.0 and
24.1 seconds, respectively.  The lot was divided out into
25   1.0 ml portions which were placed into individual vials and
lyophilized in a conventional manner.  A vial of the P1
control plasma was reconstituted the next day with distilled
water, whereafter it demonstrated a PT value of 11.0 seconds
and an APTT value of 24.1 seconds.  Similarly reconstituted
30   P1 vials were allowed to stand at room temperature (about
25°C) for up to five days, one being tested each day for PT
and APTT values.  The results are given in TABLE 1 below,
and demonstrate excellent stability.

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TABLE 1

| Day | 2 | 3 | 4 | 5 |
|-----|-----|-----|-----|-----|
| PT | 11.2 | 10.8 | 11.7 | 11.2 |
| APTT | 25.7 | 26.2 | 27.1 | 28.3 |

5　　　Equally surprising stabilities for Factors V and VIII
were shown in the P1 samples. Immediately after
reconstitution, P1 samples exhibited Factor V and VIII
values of approximately 1000% and 110%, respectively, of the
amounts found in normal human plasma pools. After
10　reconstituted samples were allowed to stand for five days at
room temperature, they demonstrated a Factor V value of
approximately 1100% and a Factor VIII value of approximately
90%.

EXAMPLE 3

15　　　*Non-Primate Based Abnormal Range Control (Level 2)*
A second pilot lot (P2) of control plasma was prepared
by combining 175 ml adsorbed pig plasma, 5 ml beef plasma,
and 20 ml of the applicant's preferred preservative solution
prepared as in Example 1 above. This lot was prepared so as
20　to have abnormal clotting times, with initial PT and APTT
values being 21.1 and 40.8, respectively. The P2 lot was
divided and lyophilized in the same manner as the P1 lot
described in Example 2 above. After reconstitution with
distilled water, a vial of the P2 lot produced a PT value of
25　20.1 and a APTT value of 41.4 seconds.
Analogous to the reconstituted stability tests of
Example 2, several reconstituted P2 vials were allowed to
stand at room temperature for up to five days with one being
tested each day for PT and APTT values. TABLE 2 below sets
30　forth the results which again demonstrate superior stability.

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TABLE 2

| Day | 2 | 3 | 4 | 5 |
|-----|-----|-----|-----|-----|
| PT | 20.6 | 22.0 | 21.6 | 22.7 |
| APTT | 43.8 | 46.1 | 44.1 | 44.4 |

## EXAMPLE 4

### Non-Primate Based Abnormal Range Control (Level 3)

A third pilot lot (P3) of control plasma was prepared by combining 90 ml adsorbed (with aluminum hydroxide gel) pig plasma with 10 ml of the applicant's preferred preservative solution prepared in accordance with Example 1 above. Initial PT and APTT values of 41.3 and 69.9 were observed for this P3 material. The P3 material was then divided and lyophilized as were the P1 and P2 lot materials of Examples 2 and 3 above. After reconstitution and storage at room temperature for about one day, a vial of P3 material was analyzed. The PT and APTT values for this reconstituted P3 plasma were 38.1 and 64.1, respectively, showing good stability.

## EXAMPLE 5

### Human/Non-Primate Normal Range Control

36 ml normal human plasma and 36 ml bovine plasma were combined with 18 ml rabbit plasma and 10 ml of the preservative solution of Example 1 to form a fourth pilot lot (P4) of control material. Initial PT and APTT values were in the normal range at 11.4 and 25.7, respectively. This P4 plasma also demonstrated good stability. A reconstituted sample allowed to stand at room temperature for 7 days registered a PT value of 11.5 and an APTT value of 29.7.

## EXAMPLE 6

### Sensitivity to Reagent Variations

In this Example, the sensitivity of the applicant's

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preferred control plasmas to variations in clotting time
reagents was compared to that of fresh, normal plasma, and
to that of CITROL I, a commercially available control plasma
marketed by American Dade.  The PT test was used in this
5  determination.  To perform the study, reagents consisting of
100, 80, 60, 40, 20, and 10% thromboplastin were prepared by
diluting the thromboplastin using 0.025 M HEPES buffer, Ph
7.35 containing 0.154 M sodium chloride.  These reagent
dilutions were then used to evaluate the PT values for the
10 three test plasmas.  The results are given in TABLE 3
below.  As can be seen, the applicant's P1 control plasma
material demonstrated significantly greater variation in PT
values resulting from a greater sensitivity to the
thromboplastin dilutions.  Thus this increase in sensitivity
15 allows detection of defective reagents more readily than
even fresh plasma.

## TABLE 3

|  | %<br>Thromboplastin | PT/Fresh<br>Normal Plasma | PT/<br>CITROL I | PT/<br>P1 Plasma |
|---|---|---|---|---|
| 20 | 100 | 11.5 | 11.4 | 11.3 |
|  | 80 | 11.6 | 11.8 | 12.1 |
|  | 60 | 12.4 | 12.7 | 13.3 |
|  | 40 | 13.5 | 13.7 | 14.9 |
|  | 20 | 15.9 | 16.4 | 18.3 |
| 25 | 10 | 18.8 | 19.5 | 22.6 |

## EXAMPLE 7

*Preferred Collecting Solution*

100 ml of the applicant's preferred collecting solution
were prepared by mixing 500 U aprotinin, about 6.25 g HEPES

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hemi sodium and 2.94 g sodium citrate, and adding distilled
water to 100 ml.  This preparation resulted in 100 ml of
collecting solution which contained:

        (a)   0.25 M HEPES hemi sodium

5       (b)   2.94 gm sodium citrate

        (c)   500 U aprotinin


  Preferably, about 9 parts by volume of blood has been
collected into about 1 part by volume of this preferred
collecting solution.


10                      EXAMPLES 8-11

                *Direct Collection into Collecting Solution*

     In order to obtain a control plasma which has high and
stable levels of coagulation Factors, particularly Factors V
and VIII, Examples 2-6 are repeated except the plasma used

15  is derived from blood which has been collected directly from
the animal source into the applicant's preferred collecting
solution of Example 7, and, after removal of red blood
cells, sucrose, additional HEPES hemi sodium, as well as
thiomersal and sodium azide are added in amounts to achieve

20  the same respective levels as in Examples 2-6.  The initial
Factor V and VIII activity values are tested and are found
to be particularly high as compared to plasmas which are
collected into simple conventional citrated anticoagulant
solutions.  Additionally, the Factor V and VIII values are

25  stabilized by the buffering and preservative solution, as is
borne out by high Factor V and VIII values obtained after
lyophilization and reconstitution, and after reconstituted
control plasma samples are allowed to stand at 37°C for
eight hours, and at room temperature for about 3 days.  PT

30  and APTT values are similarly stable upon testing after
reconstituted control plasmas are subjected to these
temperature/time conditions.

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## EXAMPLE 12

### *Adding Stable Purified Factors*

Pooled normal human plasma was assayed and demonstrated respective APTT and PT values of 34 secs and 13 secs. In various experiments, purified factors X and VIII from pig, cow, horse, sheep or rabbit were added to about 1 ml samples of the normal human plasma and in each instance corrected APTT to 26 secs. Similarly, purified Factor II from pig, cow, horse, sheep, or rabbit was added to about 1 ml samples of the normal human plasma and corrected PT to 11 secs. Additionally, in each instance, the lyophilized product showed better stability than the lyophilized normal human plasma.

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WHAT IS CLAIMED IS:

    1.   A stable coagulation control plasma, comprising:
blood plasma; and
effective amounts of:
5       a buffer to maintain a physiological pH;
a protease inhibitor; and
a suitable stabilizing carbohydrate.

    2.   The control plasma of claim 1 wherein said protease
inhibitor is aprotinin.

10    3.   The control plasma of claim 2 wherein said buffer
is HEPES buffer.

    4.   The control plasma of claim 3 wherein said
carbohydrate is sucrose.

    5.   The control plasma of claim 4 additionally
15  comprising sodium azide.

    6.   The control plasma of claim 5 additionally
comprising thimerosal.

    7.   The control plasma of claim 6 wherein said HEPES
buffer is HEPES hemi sodium, and said HEPES buffer,
20  aprotinin, sucrose, thimerosal, and sodium azide are present
in amounts of at least about 0.08 M, 0.5 U/ml, 2 weight
percent, 0.02 weight percent, and 0.08 weight percent,
respectively.

    8.   The control plasma of claim 1, 4 or 7 in which said
25  blood plasma comprises porcine, rabbit, bovine, human,
equine, sheep or goat plasma.

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9.    The control plasma of claim 8 in which said plasma
comprises equine plasma.

10.    The control plasma of claim 8 in which said plasma
comprises rabbit plasma.

5        11.    The control plasma of claim 8 in which said plasma
comprises human plasma.

12.    The control plasma of claim 11 additionally
comprising at least one purified factor of a porcine,
bovine, rabbit, equine or sheep.

10        13.    The control plasma of claim 8 in which said plasma
comprises bovine plasma.

14.    The control plasma of claim 13 in which said plasma
additionally comprises human plasma.

15.    The control plasma of claim 13 in which said plasma
15    additionally comprises rabbit plasma.

16.    The control plasma of claim 15 in which said plasma
additionally comprises human plasma.

17.    The control plasma of claim 16 in which the total
blood plasma component of said control is constituted at
20    least substantially of said rabbit, bovine and human plasma.

18.    The control plasma of claim 17 in which the total
blood plasma component of said control consists of said
rabbit, bovine and human plasma.

19.    The control plasma of claim 18 in which said

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rabbit, bovine and human plasma are present in a volumetric
ratio of about 1:2:2, respectively.

20.   The control plasma of claim 8 in which said plasma
comprises porcine plasma.

5      21.   The control plasma of claim 20 in which said
porcine plasma is adsorbed with aluminum hydroxide gel, and
in which the total blood plasma component of said control is
constituted at least substantially of said porcine plasma.

22.   The control plasma of claim 21 in which the total
10   blood plasma component of said control consists of said
porcine plasma.

23.   The control plasma of claim 20 in which said plasma
additionally comprises bovine plasma.

24.   The control plasma of claim 23 in which the total
15   blood plasma component of said control is constituted at
least substantially of said porcine and bovine plasma.

25.   The control plasma of claim 24 in which the total
blood plasma component of said control consists of said
porcine and bovine plasma.

20      26.   The control plasma of claim 25 in which said
porcine plasma is adsorbed with aluminum hydroxide gel, and
said porcine and bovine plasma are present in a volumetric
ratio of about 35:1, respectively.

27.   The control plasma of claim 20 in which said plasma
25   additionally comprises rabbit plasma.

28.   The control plasma of claim 27 in which said plasma
additionally comprises bovine plasma.

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29. The control plasma of claim 28 in which the total
blood plasma component of said control is constituted at
least substantially of said porcine, bovine and rabbit
plasma.

5        30. The control plasma of claim 29 in which the total
blood plasma component of said control consists of said
porcine, bovine and rabbit plasma.

31. The control plasma of claim 30 in which said
porcine, bovine and rabbit plasma are present in a
10   volumetric ratio of about 1:6:3, respectively.

32. A coagulation control plasma having high stabilized
levels of Factor V and VIII, comprising:
         blood plasma; and
         at least about 2 weight percent of a suitable
15   carbohydrate;
         wherein said plasma is derived from blood which has been
collected directly from an animal source into a solution
containing a buffer to maintain physiological pH, a protease
inhibitor, and citrate; and
20       wherein said carbohydrate has been added after removal
of red blood cells from said blood.

33. The control plasma of claim 32 wherein said
protease inhibitor is aprotinin.

34. The control plasma of claim 33 wherein said buffer
25   is HEPES buffer.

35. The control plasma of claim 34 wherein said
solution contains HEPES hemi sodium, aprotinin, and sodium
citrate in respective amounts of at least about 0.25 M, 5
U/ml, and 3 weight percent.

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36. The control plasma of claim 35 additionally comprising:
HEPES hemi sodium buffer; and
aprotinin;
wherein said HEPES hemi sodium buffer and aprotinin are present in said control in amounts of at least about 0.08 M and 0.5 U/ml, respectively.

37. The control plasma of claim 36 wherein said carbohydrate is sucrose.

38. The control plasma of claim 37 additionally comprising sodium azide.

39. The control plasma of claim 38 additionally comprising thimerosal.

40. The control plasma of claim 39 in which the total plasma component of the control is constituted substantially of non-primate plasma.

41. The control plasma of claim 40 in which the non-primate plasma comprises porcine plasma.

42. The control plasma of claim 40 in which the non-primate plasma comprises bovine plasma.

43. The control plasma of claim 40 in which the non-primate plasma comprises rabbit plasma.

44. The control plasma of claim 40 in which the non-primate plasma comprises equine plasma.

45. The control plasma of claim 41 in which the non-primate plasma additionally comprises bovine plasma.

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46. The control plasma of claim 45 in which the
non-primate plasma additionally comprises rabbit plasma.

47. The control plasma of claim 42 in which the
non-primate plasma additionally comprises rabbit plasma.

5       48. The control plasma of claim 41 or 45 in which the
porcine plasma is adsorbed with aluminum hydroxide gel.

49. The control plasma of claim 45 in which the porcine
plasma is adsorbed with aluminum hydroxide gel, and the
porcine and bovine plasma are present in a volumetric ratio
10   of about 35:1, respectively.

50. The control plasma of claim 46 in which the
porcine, bovine, and rabbit plasma are present in a
volumetric ratio of about 1:6:3, respectively.

51. A process for producing a stable coagulation
15   control plasma which comprises the sequential steps of:
        collecting the blood containing the plasma to be used in
the control directly from an animal source into a solution
containing a buffer effective to maintain a physiological
pH, and effective amounts of a protease inhibitor;
20       removing red blood cells from said blood; and
        adding at least about 2 weight percent of a suitable
stabilizing carbohydrate.

5     The process of claim 51 in which in said collecting
step, said buffer comprise: HEPES buffer, said protease
25   inhibitor comprises aprotinin, and a citrate is additionally
included.

53. The process of claim 52 in which in said collecting

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step, said solution comprises HEPES hemi sodium, aprotinin,
and sodium citrate in amounts of at least about 0.25 M, 5
U/ml, and 3 weight percent, respectively.

54.  The process of claim 52 or 53 in which in said
adding step, said carbohydrate is sucrose.

55.  The process of claim 54 additionally comprising the
step of:
    adding sodium azide in amounts to achieve at least about
0.08 weight percent sodium azide in said control.

56.  The process of claim 55 additionally comprising the
step of:
    adding thimerosal in amounts to achieve at least about
0.02 weight percent thimerosal in said control.

57.  The process of claim 51 in which in said collecting
step, said blood is non-primate blood; and about 9 parts by
volume of said blood are collected into about 1 part by
volume of said solution.

58.  A process for producing a stable coagulation
control comprising the steps of:
    providing blood plasma; and
    adding to said plasma at least one stable purified
coagulation factor.

59.  The process of claim 58 wherein said blood plasma
is human plasma, and said factor is Factor II, V, VIII or X
derived from a non-primate animal.

60.  The process of claim 58 wherein said factor is
Factor V or VIII derived from a bovine or porcine.

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61.   The process of claim 60 wherein said plasma is human plasma.

62.   The process of claim 60 wherein said plasma is equine plasma.

5      63.   The process of claim 60 wherein said factor is Factor V.

64.   The process of claim 58 wherein said factor is Factor II derived from an equine.

65.   The process of claim 58 wherein said factor is
10   derived from blood which has been collected directly from an animal source into a solution containing buffer effective to maintain a physiological pH, a protease inhibitor, and citrate.

# INTERNATIONAL SEARCH REPORT

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5):   G01N 31/00
U.S. Cl.:   436/16

**II. FIELDS SEARCHED**

| Minimum Documentation Searched ⁴ | |
|---|---|
| Classification System | Classification Symbols |
| U.S. | 436/8,9,10,11,12,13,14,15,16,17,18;   252/408.1 |

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

**III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴**

| Category * | Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷ | Relevant to Claim No. ¹⁸ |
|---|---|---|
| Y | US, A, 4,409,334 (Lill et al.) 11 October 1983, see the entire document. | 1-65 |
| Y | US, A, 4,234,682 (Bartl et al.) 18 November 1980, see the entire document. | 1-65 |
| Y | US, A, 4,056,484 (Heimburger et al.) 01 November 1977, see the entire document. | 1-65 |

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search ² | Date of Mailing of this International Search Report ² |
|---|---|
| 16 October 1990 | 0 6 DEC 1990 |
| International Searching Authority ¹ | Signature of Authorized Officer ¹⁰ |
| ISA/US | T. J. Wallen |